Fig. 1

Fig. 1 is a table showing the emission wavelength maximum (nm) of aequorin mutants with coelenterazine analogues.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S (Aeq3)	Aequorin Mutant S Ser5Cys (Aeq5)	Aequorin Mutant S Tyr132Ile
CTZ i	472	491	491	487
CTZ ip	472	470	454	453
CTZ h	472	476	471	471
CTZ hcp	472	476	448	465
CTZ cp	472	470	456	457
CTZ fcp	472	466	471	471
CTZ f	472	490	473	471
CTZ n	472	487		
CTZ native	472	474	471	471

* All values, except wild type aequorin, were calculated from the average of 3 trials (wild type with 2). All mutants were, except Aeq5 purified to >95% purity. CTZ analogues diluted to 100 micrograms/ml methanol.

Fig. 2

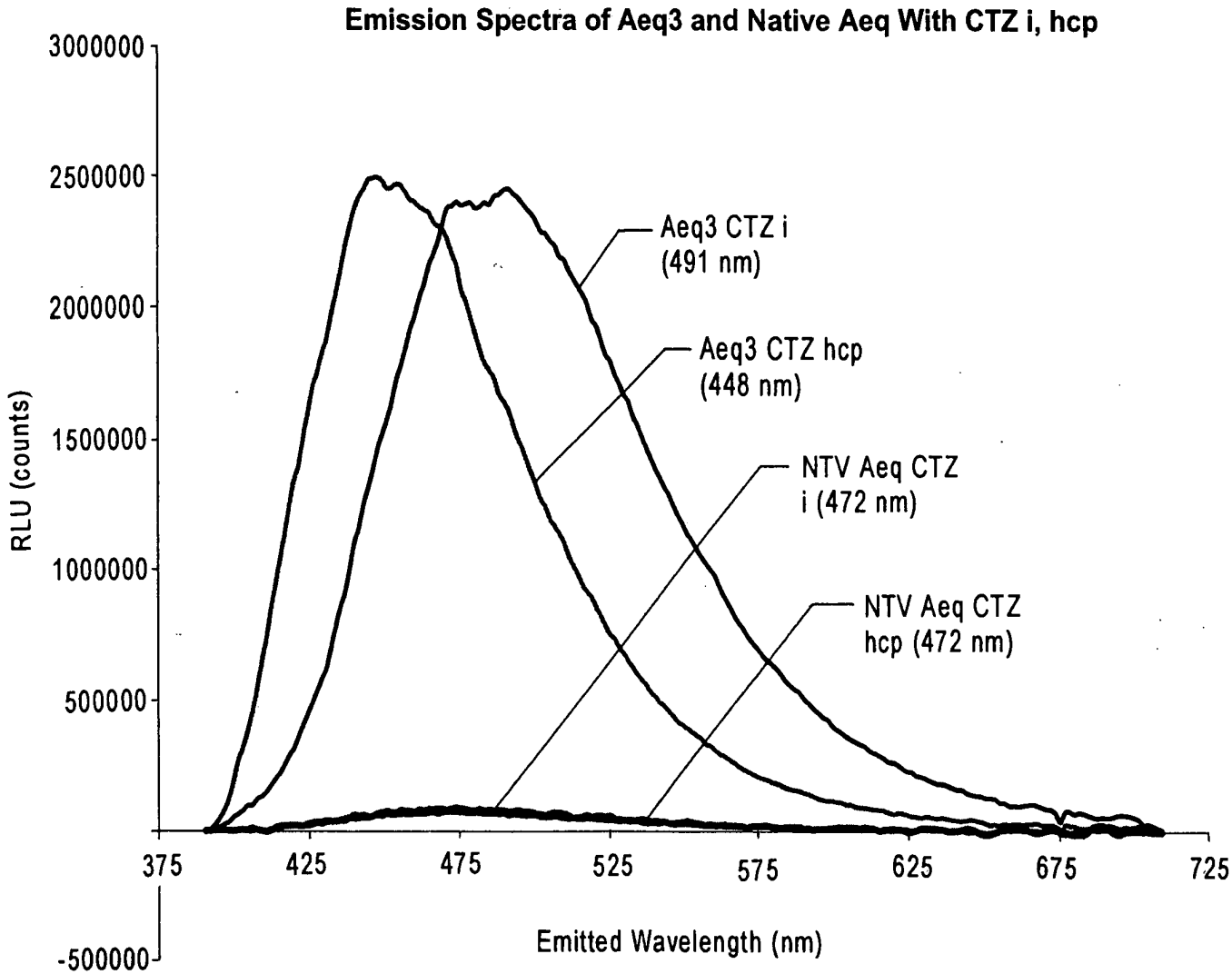

Emission Spectra of Aeq3 and Native Aeq With CTZ i, hcp
3000000
2500000
2000000
1500000
1000000
500000
-500000
RLU (counts)
375
425
475
525
575
625
675
725
Emitted Wavelength (nm)
Aeq3 CTZ i (491 nm)
Aeq3 CTZ hcp (448 nm)
NTV Aeq CTZ i (472 nm)
NTV Aeq CTZ hcp (472 nm)

Fig. 3

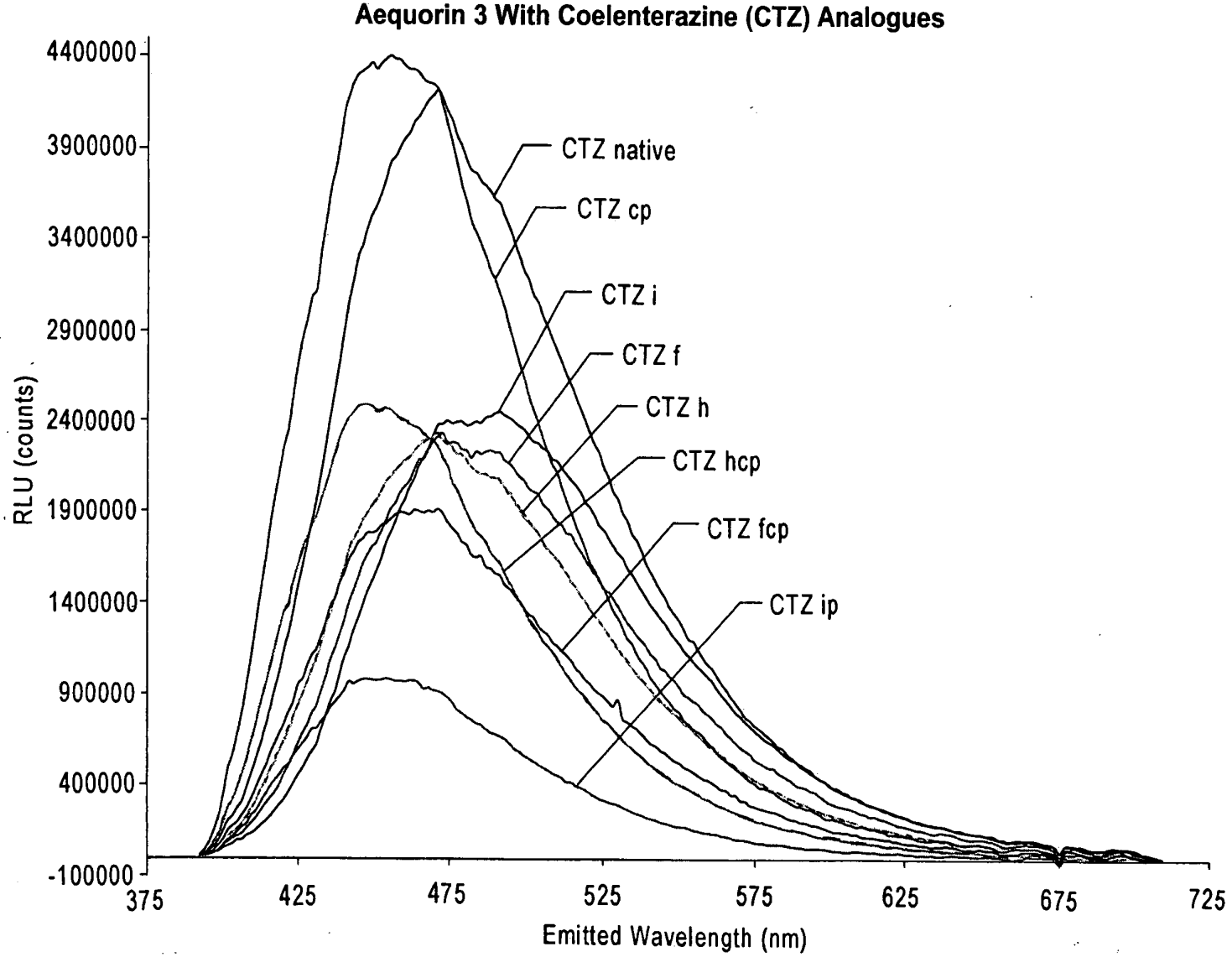

Aequorin 3 With Coelenterazine (CTZ) Analogues
4400000
3900000
3400000
2900000
2400000
1900000
1400000
900000
400000
-100000
RLU (counts)
375
425
475
525
575
625
675
725
Emitted Wavelength (nm)
CTZ native
CTZ cp
CTZ i
CTZ f
CTZ h
CTZ hcp
CTZ fcp
CTZ ip

Fig. 4

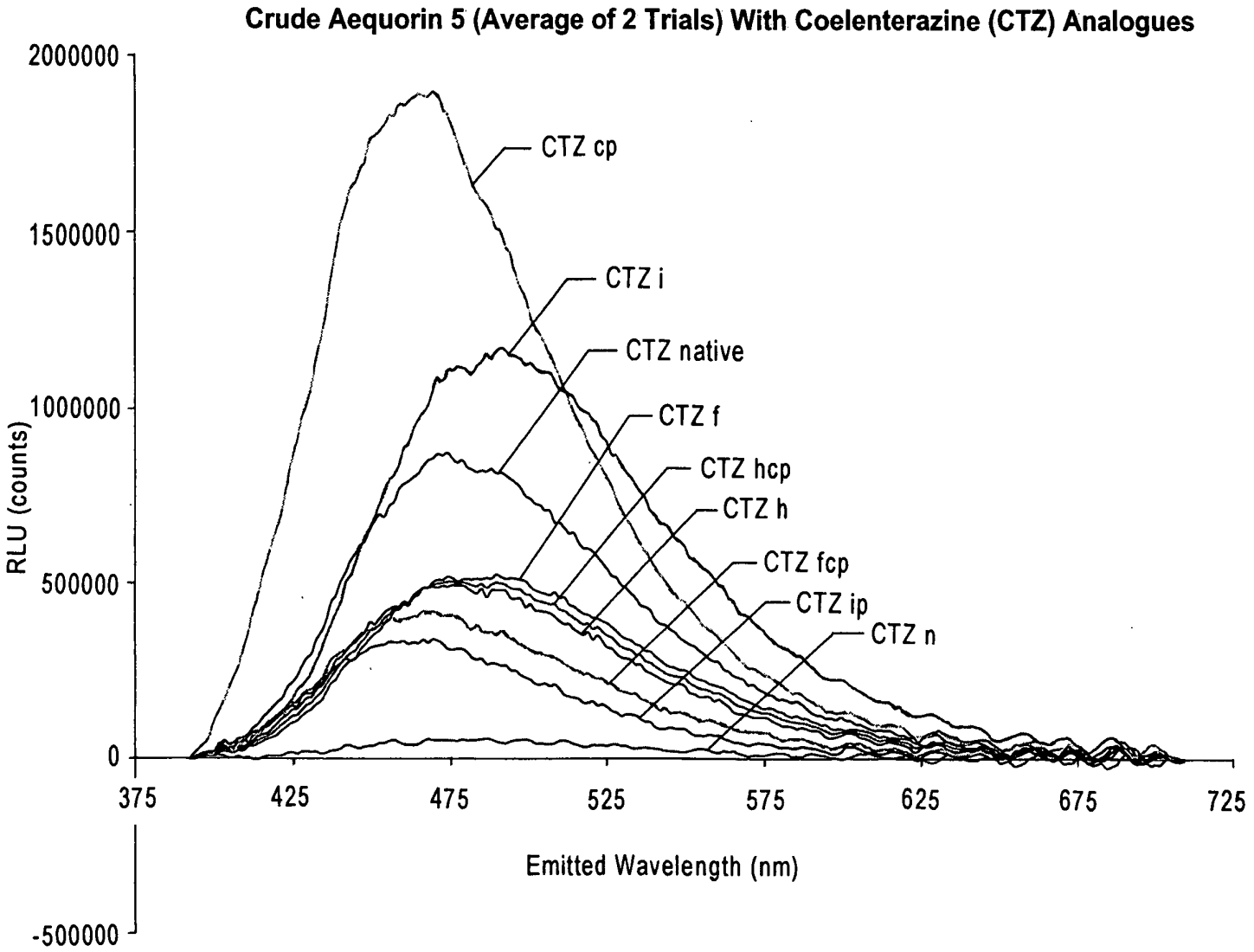

Crude Aequorin 5 (Average of 2 Trials) With Coelenterazine (CTZ) Analogues
2000000
1500000
1000000
500000
0
-500000
RLU (counts)
CTZ cp
CTZ i
CTZ native
CTZ f
CTZ hcp
CTZ h
CTZ fcp
CTZ ip
CTZ n
375
425
475
525
575
625
675
725
Emitted Wavelength (nm)

Fig. 5

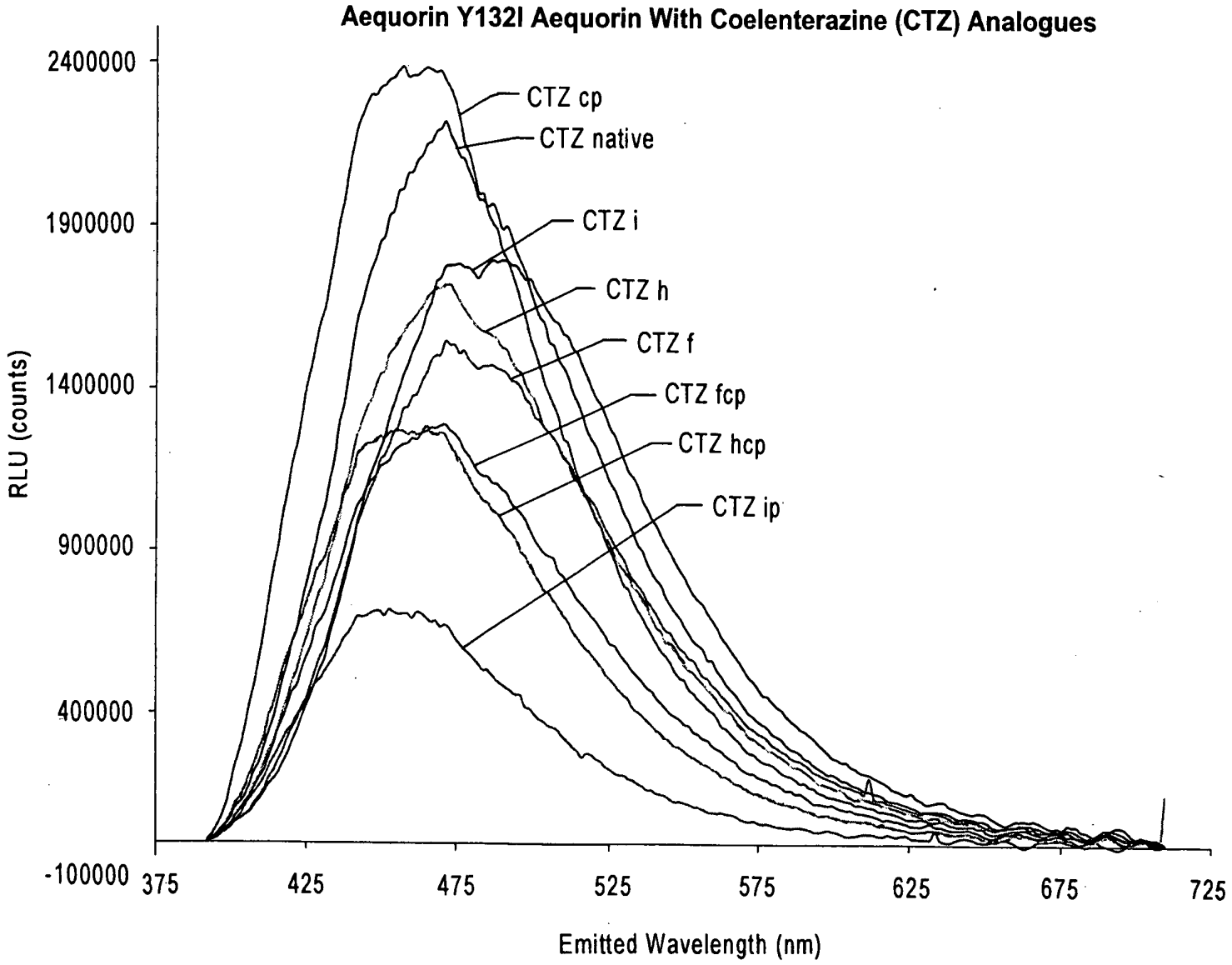

Aequorin Y132I Aequorin With Coelenterazine (CTZ) Analogues
2400000
1900000
1400000
900000
400000
-100000
RLU (counts)
375
425
475
525
575
625
675
725
Emitted Wavelength (nm)
CTZ cp
CTZ native
CTZ i
CTZ h
CTZ f
CTZ fcp
CTZ hcp
CTZ ip

Fig. 6

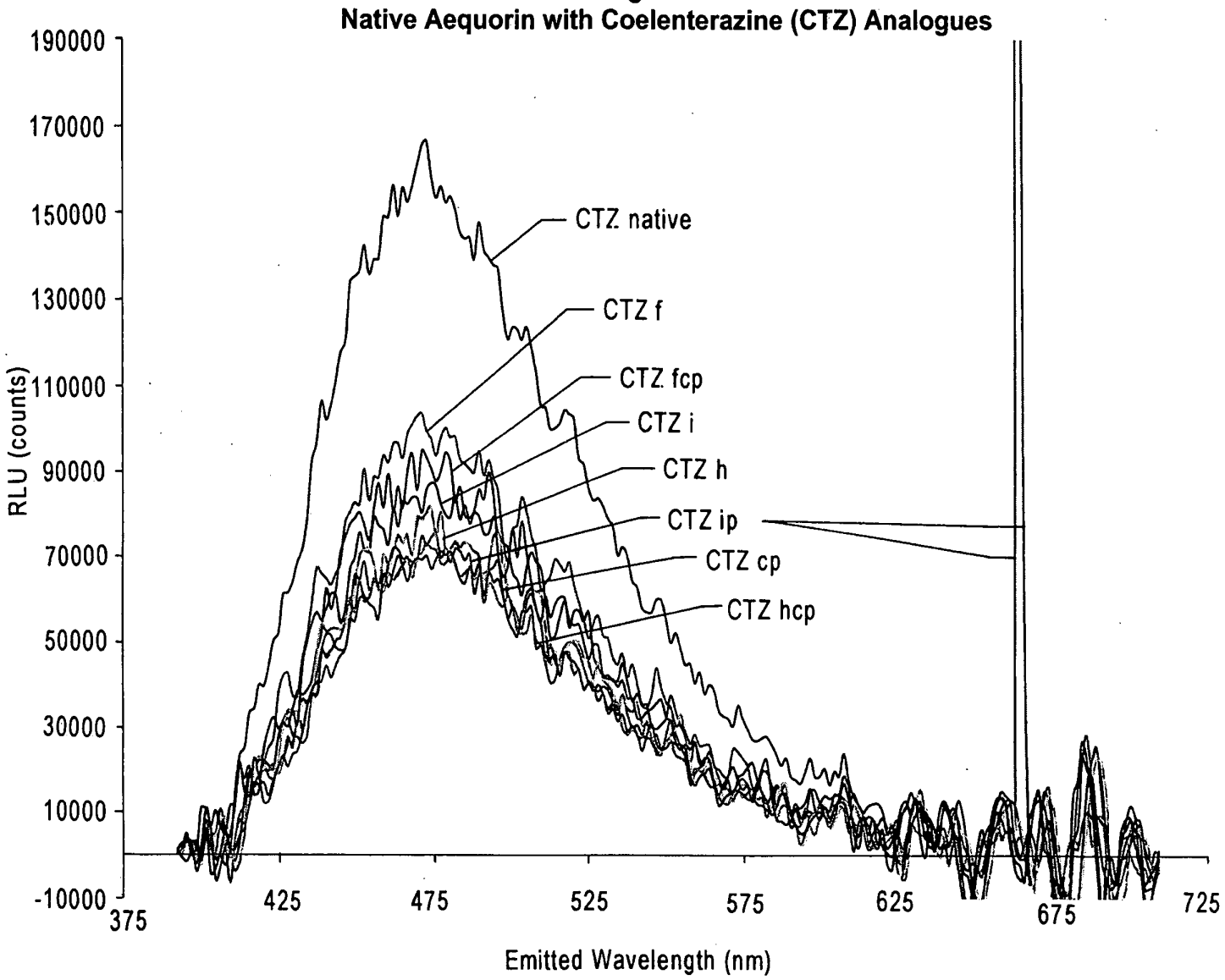

Native Aequorin with Coelenterazine (CTZ) Analogues
RLU (counts)
190000
170000
150000
130000
110000
90000
70000
50000
30000
10000
-10000
375
425
475
525
575
625
675
725
Emitted Wavelength (nm)
CTZ native
CTZ f
CTZ fcp
CTZ i
CTZ h
CTZ ip
CTZ cp
CTZ hcp

Fig. 7

Fig. 7 is a table showing the emission wavelength maximum (nm) of aequorin mutant Mutant S Y132I, Mutant S having a 3-fluoro-1-tyrosine aequorin or a 5-fluoro-1-tyrosine non-natural amino acid in position 132 in conjunction with coelenterazine analogues CTZ i, ip, n, h, hcp, cp, fcp, f and native CTZ.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S Tyr132Ile	Aequorin Mutant S Tyr132 3-fluoro-l-tyr	Aequorin Mutant S Tyr132 5-fluoro-l-trp
CTZ i	472	491	511	495
CTZ ip	472	452	471	
CTZ n	472	491	500	
CTZ h	472	472	498	471
CTZ hcp	472	452	471	468
CTZ cp	472	457	471	471
CTZ fcp	472	463	471	
CTZ f	472	472	500	497
CTZ native	472	471	495	472

Fig. 8

Spectra of 3-Fluoro-L-Tyrosine

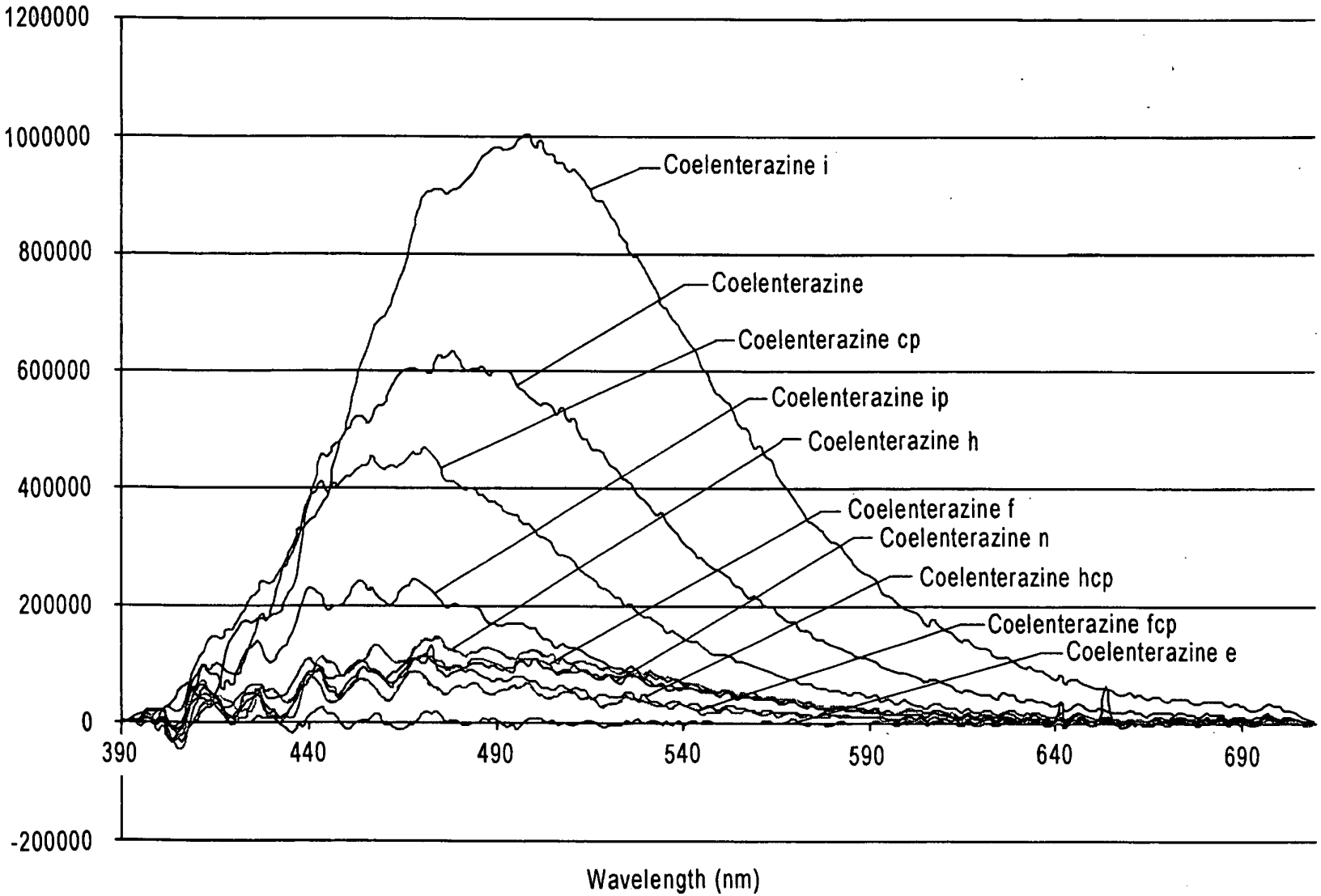


Fig. 9

Spectra of 5-Fluoro-L- Tryptophan

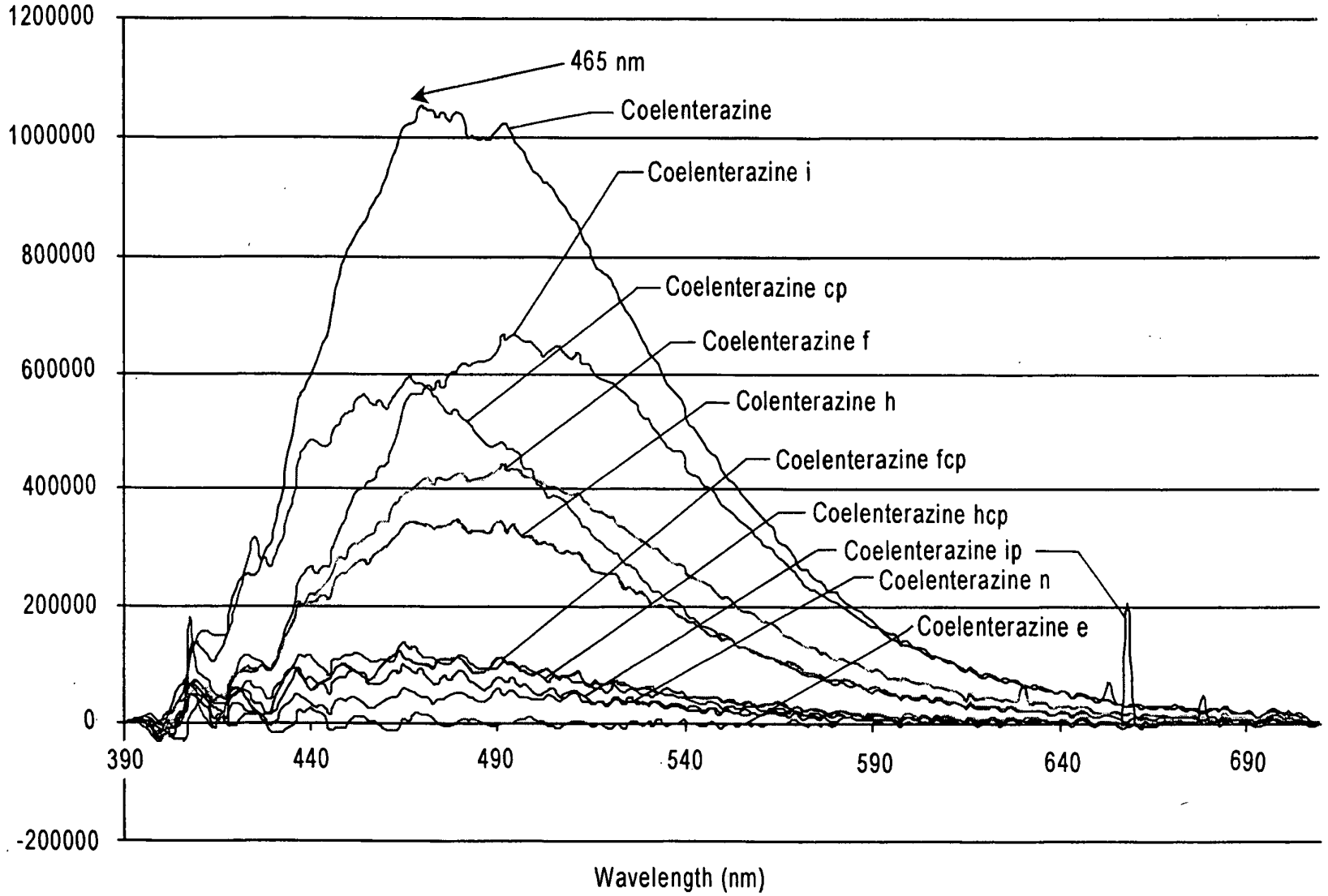


Fig. 10

Energy Transfer Study: Aequorin Mutants

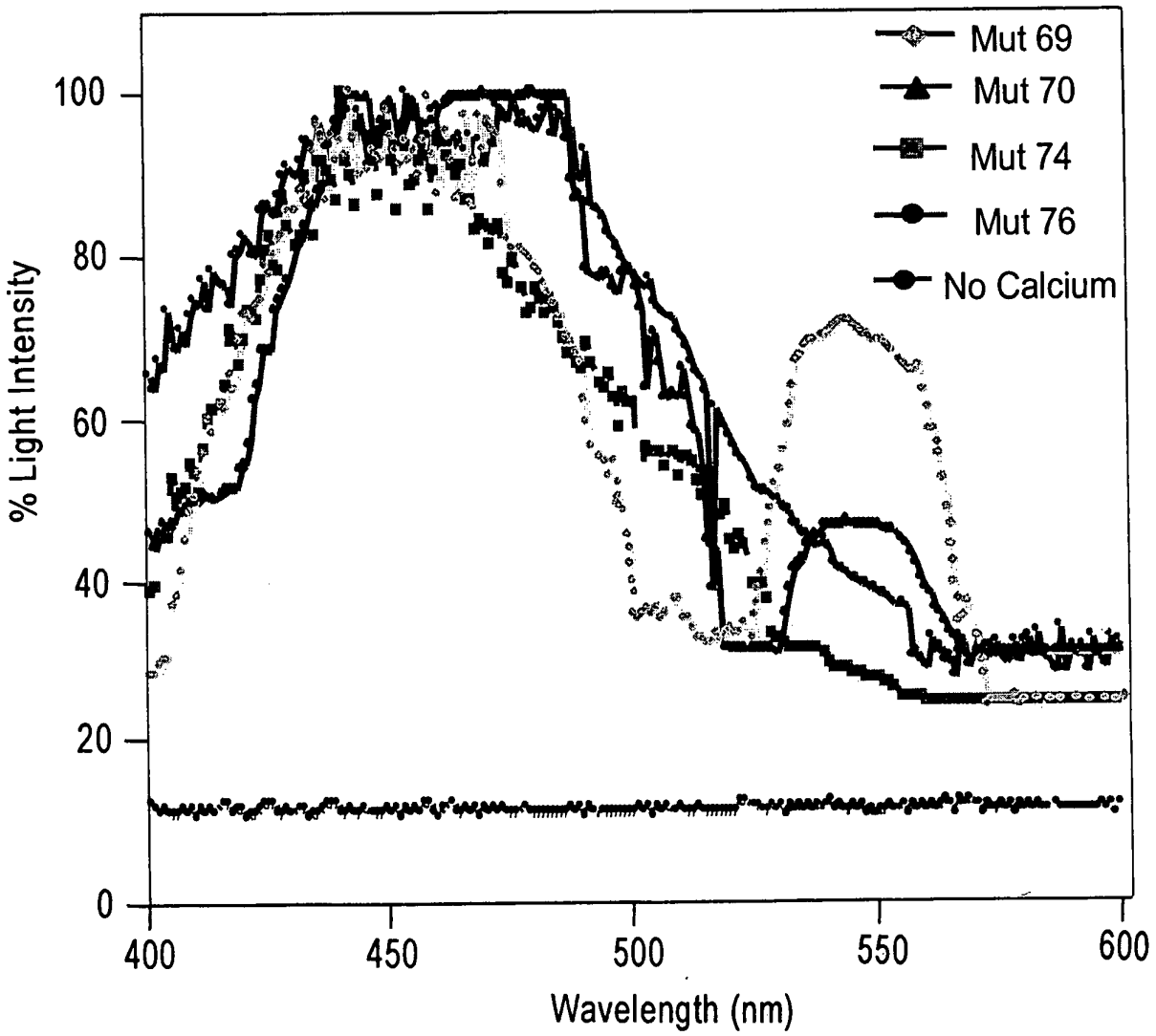


Fig. 11

SEQ ID NO: 1 cDNA encoding wild type apoaequorin

```
        1 aatgcaattc atctttgcat caaagaatta catcaaatct
ctagttgatc aactaaattg
       61 tctcgacaac aacaagcaaa catgacaagc aaacaatact
cagtcaagct tacatcagac
      121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
      181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
      241 aaccttggag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
      301 ggaggagctg gaatgaaata tggtgtggaa actgattggc
ctgcatatat tgaaggatgg
      361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gctcatccgt
      421 atatggggtg atgctttgtt tgatatcgtt gacaaagatc
aaaatggagc cattacactg
      481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agattgcgag
      541 gaaacattca gagtgtgcga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
      601 agacaacatt taggattttg gtacaccatg gatcctgctt
gcgaaaagct ctacggtgga
      661 gctgtccccc aagaagctct acggtggtga tgcaccctgg
gaagatgatg tgattttgaa
      721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
      781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
      841 gaacttacaa atcgaaaaag t
```

Fig. 12

SEQ ID NO: 2 amino acid sequence for wild type apoaequorin

```
VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVYKA SDIVINNLGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDCEETFR
VCDIDESGQL DVDEMTRQHL GFWYTMDPAC EKLYGGAVP
```

Fig. 13

SEQ ID NO: 3 cDNA encoding Mutant S apoaequorin

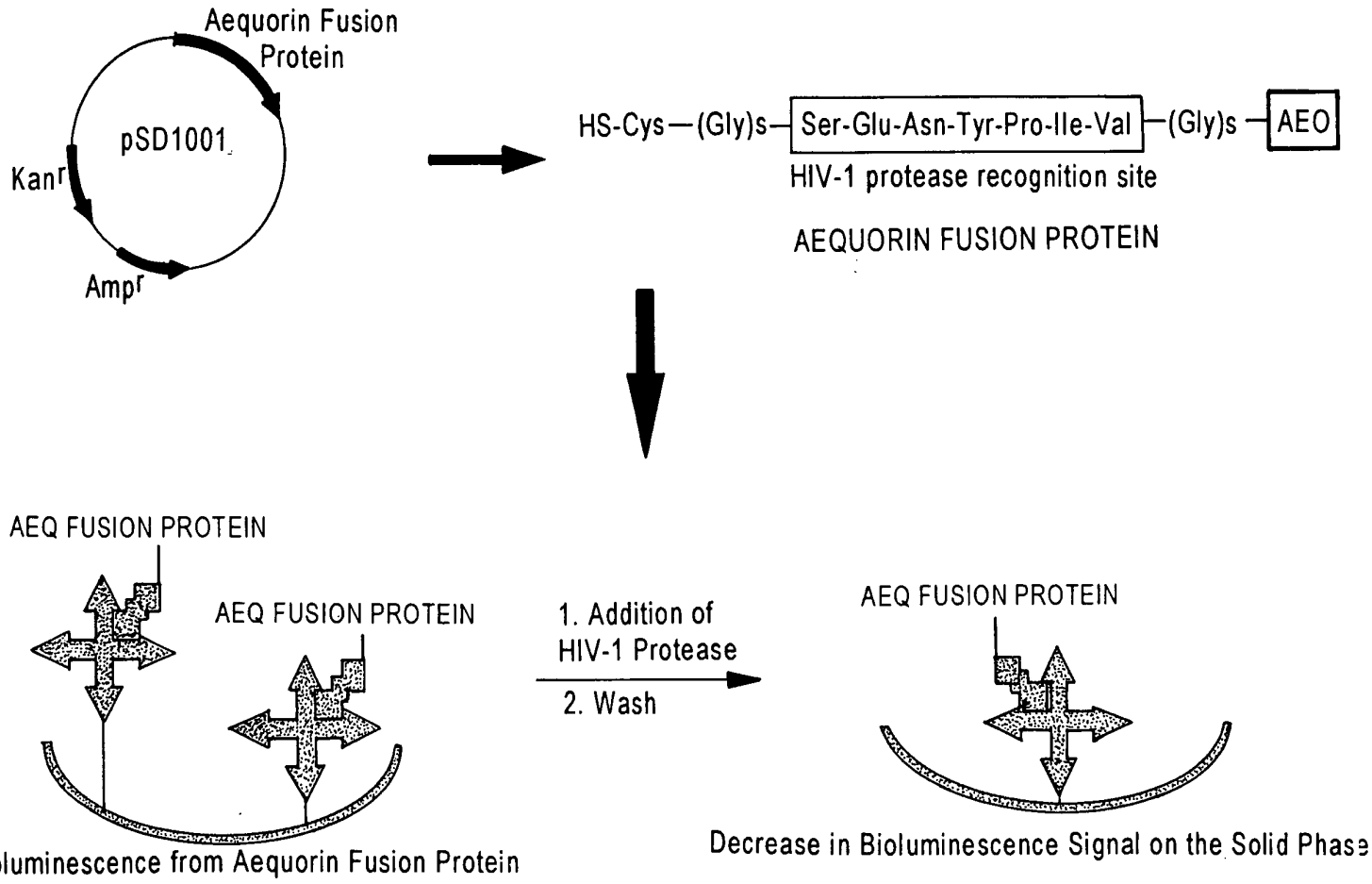
1 a**atg**caattc atctttgcat caaagaatta catcaaatct
ctagttgatc aactaaattg
61 tctcgacaac aacaagcaaa c**atg**acaagc aaacaatact
cagtcaagct tacatcagac
121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttggag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tggtgtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gctcatccgt
421 atatggggtg atgctttgtt tgatatcgtt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agat**agc**gag
541 gaaacattca gagtg**agc**ga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgct**a**
gcgaaaagct ctacggtgga
661 gctgtccccc**t** **aa**gaagctct acggtggtga tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
841 gaacttacaa atcgaaaaag t

Fig. 14

SEQ ID NO: 4 amino acid sequence for "Mutant S" apoaequorin

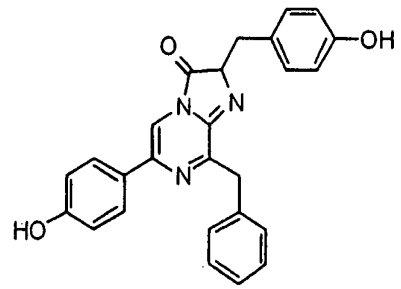
VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVYKA SDIVINNLGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSED**S**EETFR
V**S**DIDESGQL DVDEMTRQHL GFWYTMDPA**S** EKLYGGAVP

Fig. 15

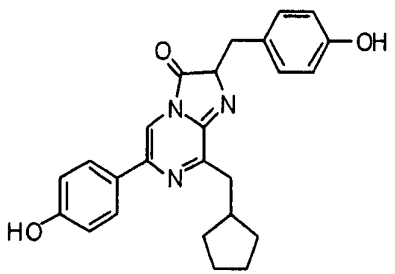


Plasmid construct for the expression of the aequorin fusion protein and schematic representation of the fusion protein showing the HIV-1 protease cleavage site. B represents biotin and NA represents Neutravidin immobilized on the wells.

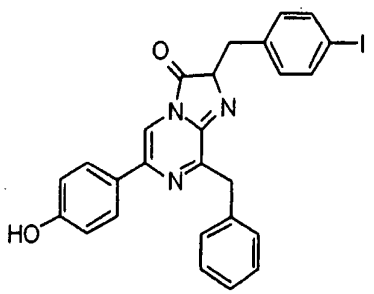
Fig. 16 Coelenterazine Analogs



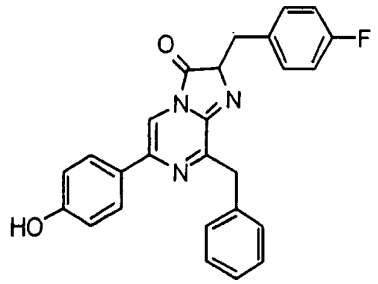
coelenterazine



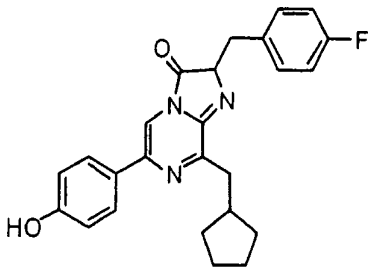
coelenterazine cp



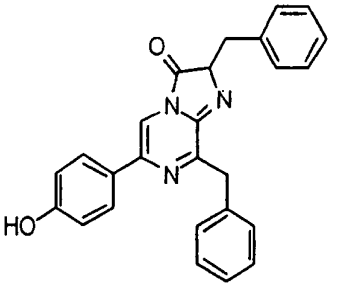
coelenterazine i



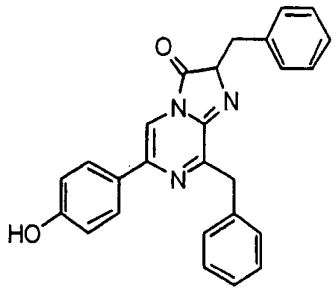
coelenterazine f



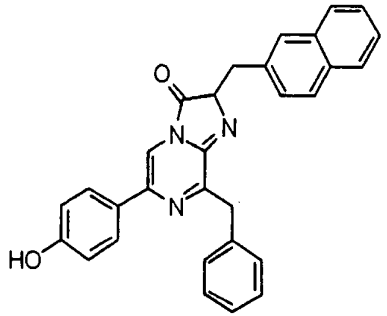
coelenterazine fcp



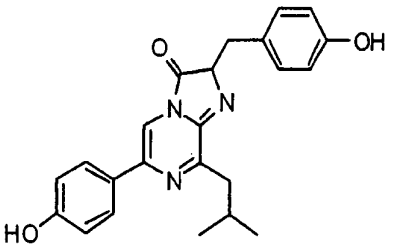
coelenterazine hcp



coelenterazine h



coelenterazine n



coelenterazine ip